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Optimization of protocol for large-scale propagation of cv. *Patakapura*: promising local cultivar of banana plant of Odisha

Vijya Laxmi and Souvagyalaxmi Sahoo

Abstract
Banana cv. *Patakapura* (*musa spp.*), local variety of Odisha have large commercial value. The present investigation deals with development of an efficient micro propagation protocol for banana cv. *Patakapura* using shoot tip from young suckers as explant. Types of cytokinin and auxin and their concentration significantly influenced the initial growth, multiplication, shooting and rooting of banana plants *in vitro*. Maximum callus induction of banana cv. *Patakapura* was observed on MS medium supplemented with BAP (8.0 mg/l) in combination with NAA (2.0 mg/l). Callus were multiply strongly on MS medium supplemented with BAP (6.5 mg/l) in combination with NAA (1.6 mg/l). Additionally, maximum shoot proliferation and elongation were observed with BAP (5.5 mg/l) and NAA (1.2 mg/l) and better rooting response was observed in NAA (4.0 mg/l) with activated charcoal. Thus, we observed that MS medium in combination with BAP and NAA growth regulators is best for banana cv. *Patakapura*. After hardening and acclimatization the plantlets were transferred to the field and showed ~75-85 % survival.

Keywords: Micropropagation, Banana, *Patakapura*, Murashige and Skoog (MS) medium Benzylaminopurine (BAP), Naphthalene acetic acid (NAA)

Introduction
Bananas (*musa spp.*) which include both plantains and table bananas grown in the tropics, are considered as one of the most important and commercially viable food crops in the world with 102 million tons of production (FAO, 2002) [4]. They are grown in at least 150 countries, primarily for their fruit in Asia-Pacific countries (e.g.- India, China and Philippines) and contributing more than 45% of the total world production (Singh *et al.* 2011) [24]. Among the major producers, India alone accounts for 27.43% (Singh *et al.* 2011) [24]. Banana is vulnerable to a number of biotic and abiotic stresses, which limit its production, has prompted interest in the use of *in vitro* culture of banana (Novak *et al.* 1986; Mendes *et al.* 1996; Roels *et al.* 2005; Gebeyehu 2015; Suman 2017; Rajamanickam *et al.* 2018) [18, 15, 21, 7, 25, 22]. Traditionally, banana is grown as a perennial crop where the plant is allowed to produce continuous shoots from a subterranean stem. But, the yields fall after three to five years and decline rapidly after ten to fifteen years (Singh *et al.* 2011) [24]. The important factor affecting the efficiency of micropropagation of banana is the rate of *in-vitro* multiplication, due to different genomic compositions of cultivars (Hirimburegama and Gamage 1997; Muhammad *et al.* 2004) [8, 17]. In addition, the rate of multiplication also appears to be related to the degree of browning of the shoot tip tissues. Thus, *in-vitro* multiplication of *musa spp.* is cultivar-specific and influenced by culture environment (Hirimburegama and Gamage 1997) [8]. Moreover, the rate of multiplication in banana is also restricted to 5-20 suckers per plant during its growth period, which makes it difficult to obtain sufficient amount of planting material of a clone of choice (Singh *et al.* 2011) [24]. Thus, micropropagation of banana facilitates rapid production of large number of plantlets/unit time, which have higher survival rate as well as shorter harvesting period (Hirimburegama and Gamage 1997; Singh *et al.* 2011; Gebeyehu 2015) [8, 24, 7]. The first reports of *in vitro* culture of bananas done in Taiwan in the 70 century (Ma and Shii 1974; Ma *et al*. 1978) [12, 13]. *In vitro* multiplication of banana plantlets has been achieved using shoot tip (Cronauer and Krikorian 1984; Novak *et al.* 1986; Vuylsteke 1989; Nguyen and Kozai 2001) [1, 18, 28, 20], from male floral apices (Escalant *et al.* 1994) [3] and from somatic embryogenesis (Novak *et al*. 1989) [19]. In India, different cultivars of banana plants is grown under different climatic condition, such as *Dwarf Cavendish*, *Robusta*, *Monthan*, *Poovan*, *Nendran*, *Red*

banana, *Nyali*, *Safed Velchi*, *Basarai*, *Ardhapuri*, *Rasthali*, *Karpurvalli*, *Karthali* and *Grandnaine* etc. (Govil and Gupta 1997; Singh *et al.* 2011) [6, 24]. Several researchers have reported the *in vitro* propagation of a wide range of *musa* species and cultivars (cv.) belonging to various ploidies and genomes (Sathiamoorthy *et al.* 1998; Diniz *et al.* 1999; Nauyen and Kozai 2001; Roels *et al.* 2005; Madhulatha *et al.* 2004; Kalimuthu *et al.* 2007) [23, 2, 20, 21, 16, 10]. Banana cv. *Patakapura* (*musa spp.*) mostly grown in the coastal tracts of Odisha. Among the varieties of banana grown in the state the cv. *Patakapura* is well admired for its typical flavor and aroma. In this study, we attempted to optimize the protocol for micropropagation of banana cv. *Patakapura* through multiple shoot induction for commercialization.

Materials and methods

Four-week-old suckers of selected banana cv. *Patakapura* plants were collected and peeled off. Shoot tip from young suckers of 40-100 cm, containing meristem was selected as explants. The explants were prepared by removing the outer layer of tissue from suckers with a clean knife. Then explants were washed in running tap water to remove dirty particles from the surface and surface sterilized for 15 minutes with teepol (cleaning detergent). After teepol treatment, explants were soaked in bavistin (2 gm/L) for 15 minutes and rinsed with distilled water. The ensheathing leaf bases were removed from the pseudo stem and explants were trimmed to final size of 3-5 mm in the laminar flow cabinet, leaving the young leaves around the meristem. Subsequently, banana explants were transferred into 0.2% mercuric chloride solution for 10 min and washed thrice with autoclaved distilled water. Finally, explants were transferred on filter paper for drying the excess amount of water. After sterilization, explants were inoculated aseptically in Murashige and Skoog (MS) media (Murashige and Skoog 1962) [11] alone and supplemented with 6-benzyl amino purines (BAP) and a naphthalene acetic acid (NAA) in various combinations as shown in Table 1-4. BAP and NAA concentration and ratio determines the growth and morphogenesis of banana tissue culture. *In vitro* culture of banana often suffer from excessive blackening caused by oxidation of polyphenolic compound released from wounded tissue preventing nutrient uptake and hindering growth. Therefore, antioxidant was added to reduce blackening. Callus cultures were inoculated in 25x150 mm culture bottles containing 40 ml of MS medium supplemented with different combinations of callus induction medium (Table 1) and these calli were transferred to four different multiplication medium (Table 2). For regeneration, green healthy friable calli were divided into small pieces and cultured on shoot regeneration medium (Table 3). Calli were sub cultured at regular interval of two-three weeks to avoid phenolic accumulation in banana tissues. Once the sufficient number of shoots has been generated, it was transferred to medium that contains higher concentration of auxin for root formation (Table 4). Activated charcoal was added in medium to eliminate the residual effect of cytokinin by absorption (Thomas 2008) [26]. Banana cultures were maintained at 25±2°C under 16 h photoperiod at 1000-lux light intensity. After root formation, rooted plantlets were dipped in bavistin (fungicide) to remove contaminants and adhering medium. Then it was washed with water and transferred to net pots containing a mixture of garden soil and coco pit in the ratio of 1:1. These net pots were transferred to poly house for 20 days for primary hardening. After 20 days, these plantlets were transferred to polythene bags filled with autoclaved mixture of soil and soilrite (commercial hardening mixture) and kept in green house for 3-4 months for secondary hardening. Subsequently, these fully developed banana cv. *Patakapura* plantlets were transferred to the field.

Table 1: MS medium with different hormonal concentration of BAP and NAA for initial culture.

S.N.	Culture bottle	Hormone concentration for initial culture (1000 ml of media)	
		BAP	NAA
1.	I-A	1.0 mg	0.16 mg
2.	I-B	2.0 mg	0.24 mg
3.	I-C	4.0 mg	0.10 mg
4.	I-D	8.0 mg	2.00 mg

Table 2: MS medium with different hormonal concentration of BAP and NAA for multiplication culture.

S.N.	Culture bottle	Hormone concentration for multiplication culture (1000 ml of media)	
		BAP	NAA
1.	M-A	6.0 mg	1.5 mg
2.	M-B	6.5 mg	1.6 mg
3.	M-C	7.0 mg	1.8 mg
4.	M-D	7.5 mg	1.9 mg

Table 3: MS medium with different hormonal concentration of BAP and NAA for shooting culture.

S.N.	Culture bottle	Hormone concentration for shooting culture (1000 ml of media)	
		BAP	NAA
1.	S-A	5.0 mg	1.0 mg
2.	S-B	5.5 mg	1.2 mg
3.	S-C	6.0 mg	1.5 mg
4.	S-D	6.5 mg	nil

Table 4: MS medium with different hormonal concentration of BAP and NAA for rooting culture.

S.N.	Culture bottle	Hormone concentration for rooting culture (1000 ml of media)	
		BAP	NAA
1.	R-A	nil	1.0 mg
2.	R-B	nil	2.0 mg
3	R-C	nil	2.0 mg
4.	R-D	nil	4.0 mg

Results and Discussion

In vitro multiplication of *musa spp.* is cultivar-specific and influenced by different factors (Hirimburegama and Gamage 1997) [8], thus in the present work, we optimized the culture condition for *in vitro* micropropagation of banana cv. *Patakapura* plant from shoot tip. Shoot tip culture is simple, easy and applicable to wide range *musa spp.* genotypes (Novak *et al.* 1986; Vuylsteke 1989; Nguyen and Kozai 2001) [18, 28, 20]. To achieve this goal, different concentration of BAP and NAA hormones were used alone or in combination with one another for micropropagation of banana cv. *Patakapura*. The initial best response of callus induction was observed with BAP (8.0 mg/l) in combination with NAA (2.0 mg/l). After 45 days of incubation period banana explants were completely swelled and multiple shoot bud developed from creamish green friable calli. Shoot proliferation and callus induction were occur simultaneously in banana tissue culture. Banana calli were multiply strongly in BAP-6.5 mg/l in combination with NAA (1.6 mg/l) hormonal concentration. It was reported that, there is differences in rate of multiplication of different *musa spp*. (Khatri *et al.* 1997; Vuylsteke 1998;

Muhammad *et al.* 2004) [9, 27, 17]. The shoot proliferation and elongation were stronger in BAP (5.5 mg/l) and NAA (1.2 mg/l) hormonal concentration. Among different plant growth regulators tested for root induction from regenerated shoots, NAA at a concentration of 4.0 mg/L with activated charcoal was found to give the best rooting response. Earlier also *in vitro* culture of different spp. of banana on MS medium supplemented with different concentrations of BAP and NAA had shown good regeneration response (Mendes *et al.* 1996; Gebeyehu 2015) [15, 7]. After primary and secondary hardening banana cv. *Patakapura* plantlets were transferred to the field which showed ~75-85 % survival. In the present investigation, we have established an efficient and simple protocol for the plant regeneration of banana cv. *Patakapura* using callus cultures induced from shoot tip explants. This protocol may be applied for genetic transformation in banana cv. *Patakapura* (May *et al.* 1995) [14]. Thus, micro propagation of banana is a potential method for supply of disease free and healthy planting materials at low cost over traditional propagation methods.

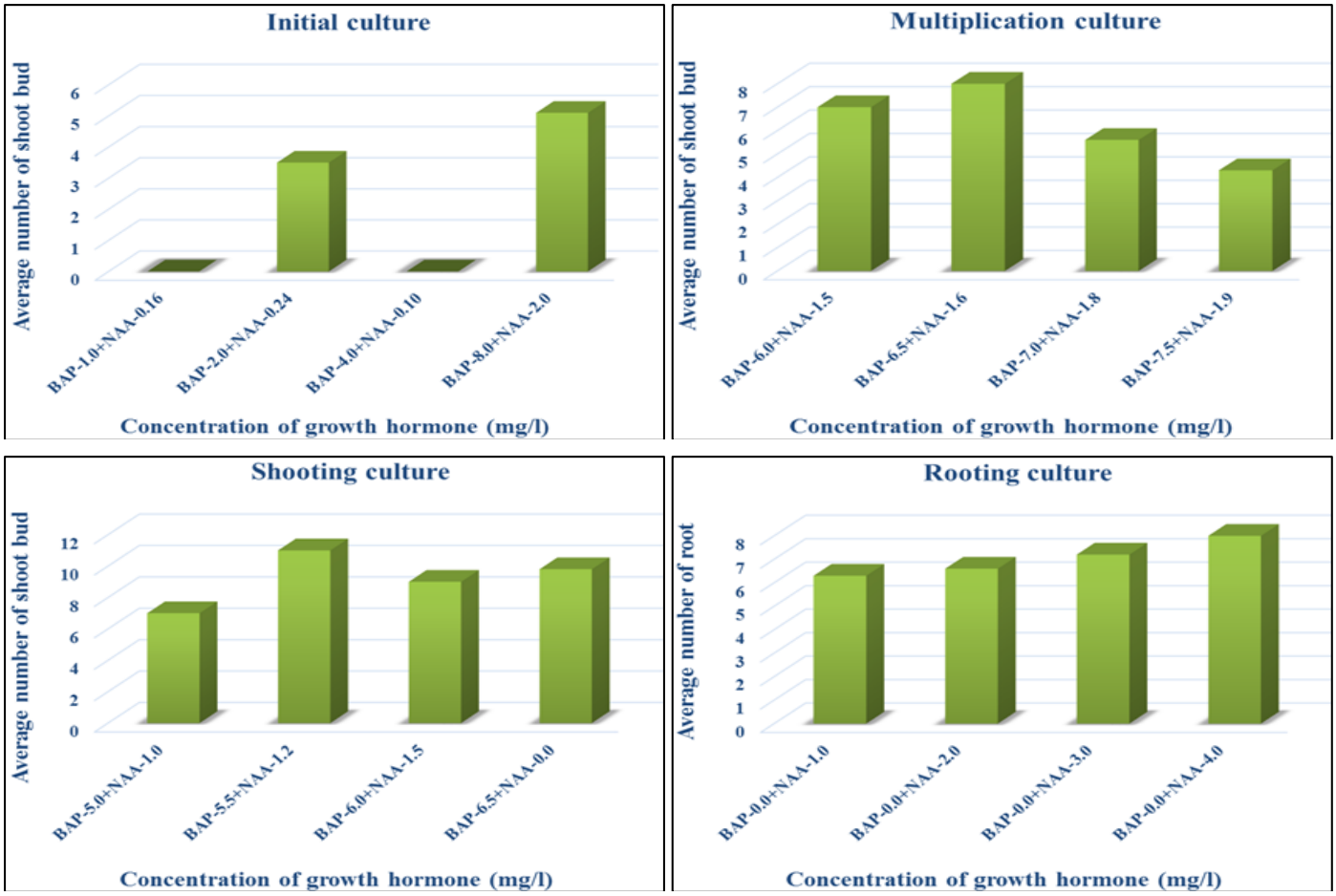

Fig 1(a, b, c, d): Influence of different concentration of BAP and NAA on (A) Initial, (B) Multiplication, (C) Shooting and (D) Rooting stages of banana cv. *Patakapura.*

Fig 2(a, b, c, d, e, f): Observation of different stages of banana cv. *Patakapura* in response to different concentration of BAP and NAA *in vitro* (A) Initial culture and (B) Callus induction and maintenance on MS medium supplemented with BAP (8.0 mg/l) in combination with NAA (2.0 mg/l), (C) Multiplication were observed with BAP (6.5 mg/l) in combination with NAA (1.6 mg/l), (D) Shoot regeneration and multiplication on MS medium supplemented with BAP (5.5 mg/l) and NAA (1.2 mg/l), (E) Rooting of regenerated shoot NAA (4.0 mg/l) with activated charcoal and (F) Two months old micropropagated plantlets established under greenhouse conditions.

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